578-Pos Board B457
Voltage-Dependent Modulation of Cardiac Ryanodine Receptors (RyR2) by Protamine
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Early studies showed that protamine (>20μg/ml) inhibits skeletal RyR1 regardless of the cytosolic Ca^{2+} levels (Koulen et al., Mol. Biol. Cell. 11:2213–9, 2000). We extended these studies to cardiac RyR2 reconstituted into planar lipid bilayers. We found that protamine (0.2μg/ml) added to the cytosolic surface of RyR2 decreased channel activity. This effect did not depend on cytosolic Ca^{2+} levels or on the presence/absence of agonists. The action of protamine was complex and involved transition to several substates as well as full block of the channel. Substates did not seem to represent protamine binding to a ryanoid site as they were observed in ryanodine-modified channels. At Vm > 0mV (SR lumen - cytosol) block events were rare and only the substate with the highest level of conductance had high probability. As expected, increasing Vm from 0 to +80mV, decreased the apparent on-rate of protamine-induced substate and increased its off-rate. Unexpectedly, the I-V relationships for the full openings and for the protamine-induced substate were parallel (i.e., the current amplitude drop induced by protamine had the same magnitude at 0 and +80mV). This is not the case with most known conductance modifiers, which induce a drop in the current amplitude that changes proportionally with the amplitude of the full opening. In contrast, the modifications in the RyR2 conduction pathway induced by protamine (conformational changes or partial occlusion of the pore) heavily depended on the electric field. Analogous electrostatic interactions between neighboring RyRs and/or with associated proteins may play a role in the heterogeneity of RyRs current amplitudes frequently observed in multichannel recordings (Supported by NIH R01 GM078665 to JAC).

579-Pos Board B458
SAM Regulation of RyR2
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RyR2 channel activity is subject to allosteric and posttranslational regulation. S-adenosyl-L-methionine (SAM), the primary methyl group donor for enzyme-mediated methylation of proteins and other biological targets, activates RyR2 via an unknown mechanism. To determine if activation of RyR2 by SAM requires methyltransferase activity, cardiac sarcoplasmic reticulum (CSR) vesicle [^{3}H]ryanodine binding was performed in media containing 150 mM KCl, 20 mM PIPES pH 7.0, 3 μM Ca^{2+}, 1 mM SAM, and 0–1 mM sinefungin, a competitive methyltransferase inhibitor. Sinefungin did not alter CSR vesicle [^{3}H]ryanodine binding, and the SAM-induced increase in [^{3}H]ryanodine binding was not altered by sinefungin. To investigate further whether activation of RyR2 by SAM involves RyR2 methylation, RyR2 was immunoprecipitated from CSR vesicles pretreated for 30 min at 37°C with 3 μCi; 285 nM [^{3}H]SAM plus or minus 500 fold excess cold SAM or 1 mM SAH, an inhibitor of methyltransferase activity, followed by centrifugation through sucrose. Radioactivity incorporated into pretreated CSR and immunoprecipitated RyR2 was determined by liquid scintillation counting. Although the amount of radioactivity incorporated into [^{3}H]SAM pretreated CSR vesicles was reduced from 6.54 to 1.86 and 1.69 pmol [^{3}H]SAM/mg protein in the presence of SAH and excess cold SAM respectively, the amount of radioactivity recovered by immunoprecipitation with anti-RyR was not increased over control (immunoprecipitation without RyR specific antibody). Because SAM contains an adenosine moiety and may activate RyR2 via interaction with the channel's adenine nucleotide binding site(s), the affects of SAM and ATP on RyR2 activity were compared. The SAM and ATP concentration dependence of CSR vesicle [^{3}H]ryanodine binding virtually overlapped with no differences at any concentration tested. This work suggests SAM does not methylate RyR2 and the similarities between ATP- and SAM-induced RyR2 activation support allosteric regulation of RyR2 by SAM.

580-Pos Board B459
Targeted Stabilisation Of The RyR2 I-Domain Restores Ca^{2+} Handling And Intercellular Synchrony In Ouabain-disrupted Cardiac Cell Monolayers
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Stabilisation of cardiac ryanodine receptors (RyR2) has emerged as an important approach for normalising Ca^{2+} cycling dysfunction in cardiac disease. Here, we targeted the I-domain of human RyR2, a region critically involved in RyR2 intramolecular rearrangement following channel activation. The I-domain is a hot-spot for arrhythmia-linked mutations that destabilise the activated channel. Putative functional motifs within the I-domain were mapped using a bioinformatics approach and the synthesised using an in vitro expression system. The efficacy of affinity-purified fragments in normalising Ca^{2+} handling and intercellular coupling was screened using a cardiac-cell model of ouabain-induced dysynchrony. Under defined experimental conditions, ouabain perturbs normal intracellular Ca^{2+} cycling and ablates the synchronous intercellular coupling in super-confluent HL-1 monolayers. A 146 amino acid fragment, termed ID^B, normalised ouabain-induced Ca^{2+} dysfunction and resulted in the re-synchronisation of Ca^{2+} transients across the monolayer. In the same ouabain-disrupted model , bacterially-synthesised ID^B increased the extent of intercellular synchrony to levels greater than those measured in naïve (non-ouabain treated) spontaneously contractile HL-1 cells. The fortuitous presence of overlapping, truncated recombinant fragments that spanned the entire ID^B sequence and co-purified with intact ID^B from bacterial culture may have contributed to these effects. Importantly, ID^B-mediated normalisation of Ca^{2+} handling and intercellular synchronisation within the ouabain-treated monolayer extended to distant cell populations that had not been transduced with the recombinant protein. In experiments using cyan-yellow bio-engineered RyR2 (CYBER) probes that report intra-RyR2 conformational rearrangement, we showed that the ID^B-mediated stabilisation of the RyR2 channel directly correlated with the normalisation of ouabain-induced Ca^{2+} dysfunction. Our data provides evidence that a specific epitope-targeting strategy can stabilise RyR2 and that this approach may have remarkable therapeutic utility in normalising channel abnormalities associated with acquired and genetic cardiac disease.

581-Pos Board B460
The Effect of Volatile Anaesthetics on the Cardiac Ryanodine Receptor
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Although volatile anaesthetics serve a crucial role in preventing pain they continue to have side effects such as their ability to excite Ca^{2+} release from the sarcoplasmic reticulum (SR) via the ryanodine receptor calcium release channels (RyR). Here we report the first detailed investigation on the effects of volatile anaesthetics on the function of cardiac RyRs.
RyRs were isolated from sheep hearts and incorporated into artificial lipid bilayers and subjected to single channel recording. Clinical doses of halothane and isoflurane increased RyR activation by luminal and cytoplasmic Ca^{2+} by increasing channel open time and opening frequency. The K_a's for halothane and isoflurane were 1 mM and 3 mM, respectively. However, the maximal effect of halothane (5-fold increase in $placeP_o$) was ~3-fold larger than that for isoflurane. These agents activated RyRs by interacting with cytoplasmic domains distinct from the ATP activating sites.
The effects of halothane on RyR regulation by luminal and cytoplasmic Ca^{2+} and Mg^{2+} were accurately fitted by a luminal-triggered calcium feedthrough model involving four Ca^{2+} sensing mechanisms on each RyR subunit; two activation sites (luminal *L*-site, 40 μM affinity; cytoplasmic *A*-site, 1 μM affinity) and two cytoplasmic inactivation sites (I_1-site, 10 mM affinity; I_2-site, 1 μM affinity). Halothane did not appear to alter the ion binding affinities for these sites. Rather, it increased channel opening rate and decreased the channel closing rate associated with Ca^{2+} binding to the two activation sites.
The potentiating effect of halothane on luminal Ca^{2+} activation of cardiac RyRs was due to 1) an increase on opening frequency because of synergistic actions of the luminal and cytoplasmic (L and A) sites and 2) an increase in open time because of Ca^{2+} feedthrough to the A-site.

582-Pos Board B461
Iron (II) Modulation of the Cardiac Ryanodine Receptor (RyR2)
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Cardiomyopathies and arrhythmias are major causes of mortality in chronic iron overload. There is evidence that iron overload impairs cardiomyocytes Ca^{2+} homeostasis (Baptista-Hon *et al*, 2005). However its molecular substrates remain unknown. Cardiac ryanodine receptors (RyR2) dysfunction is implicated in several diseases where Ca^{2+} homeostasis is lost. We therefore wanted to investigate RyR2 role as a potential target for iron-induced cardiomyopathies.
We isolated heavy sarcoplasmic reticulum (HSR) vesicles containing RyR2 from sheep hearts. RyR2 were reconstituted into L-α-phosphatidylethanolamine (PE) bilayers. Unitary currents were measured under voltage-clamp with 250mM Cs^+ as the charge carrier and 10μM activating Ca^{2+}. High affinity [^{3}H]ryanodine binding of the native vesicles was detected by liquid scintillation counting. Non-specific binding determined by incubations with 100x cold ryanodine.
Fe^{2+} reduced RyR2 open probability and conductance in a dose dependent manner. Lifetime analysis revealed 5 shut times components and 3 open times components in control. Fe^{2+} caused an extra-shut component. Furthermore, there